

Design, synthesis and preliminary evaluation of peptidomimetic inhibitors of HIV aspartic protease with an epoxyalcohol core

Fabio Benedetti,^{a,*} Federico Berti,^a Stanislav Miertus,^c Domenico Romeo,^b
Francesca Schillani,^a and Alessandro Tossi^b

^aDipartimento di Scienze Chimiche and ^bDipartimento di Biochimica, Biofisica e Chimica delle
Macromolecole, Università di Trieste, via L. Giorgieri 1, I-34127 Trieste, Italy

^cICS-UNIDO, AREA Science Park, I-34012 Trieste, Italy

E-mail: benedett@units.it

Dedicated to Professor Branko Stanovnik on his 65th birthday
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Abstract

Two peptidomimetic inhibitors based on a novel epoxyalcohol core were designed to target the epoxide ring at the catalytic aspartates of HIV-protease for irreversible inhibition of the enzyme. The inhibitors were synthesized with a multi-step approach which includes Horner-Emmons olefination of a phenylalanine-derived phosphono ketone, stereoselective reduction of the resulting *trans*-enones to allylic alcohols and *syn*-epoxidation of the latter. The epoxyalcohols thus obtained were assayed for their ability to inhibit HIV-PR and were shown to inhibit the protease with IC₅₀ values of 39 and 150 μM, respectively. This confirms that the designed epoxides are recognised with fairly good affinity by the enzyme's active site, a pre-requisite for selective irreversible inhibition.

Keywords: HIV, aspartic protease, inhibitor, epoxide, peptidomimetic

Introduction

The human immunodeficiency virus (HIV) is the causative agent of the acquired immunodeficiency syndrome (AIDS); in the last decade much effort has been devoted to the development of efficient chemotherapies that can control the progress of the viral infection.^{1,2} Inhibitors of the HIV protease (HIV-PR), the enzyme responsible for proteolytic cleavage of the viral *gag-pol* polyprotein into its functional subunits, have been shown to be effective in preventing viral maturation^{3,4} and following intense research in this field six HIV-PR inhibitors have been approved for clinical use while many more are in the pipeline and awaiting clearance.¹⁻⁵

HIV-PR is an aspartyl protease whose active form is a symmetric homodimer of a chain consisting of 99-residues. The two subunits are correlated by a C_2 axis running across the binding site, and each monomer contributes a catalytic aspartyl residue.^{6,7} The peptide substrate is recognized in an extended conformation⁸ and the aspartate diad is responsible for the catalytic acceleration through a general acid / general base mechanism.⁹ HIV-PR inhibitors used in the clinical practice are peptidomimetics that reversibly inhibit the enzyme by competing with the substrate for the active site. The availability of structural information from X-ray crystallography and previous knowledge accumulated in the development of inhibitors of other aspartic proteases have greatly facilitated the design of potent inhibitors with IC_{50} values in the nanomolar and subnanomolar range.^{3,4,9-14} A major problem in anti-AIDS therapy, however, is the rapid emergence of drug-resistant mutants, a consequence of the high rate of replication of the virus and of the high error rate of reverse transcription.^{1-4,9} Mutations at, or near, the binding site may decrease the enzyme's affinity for an inhibitor, without significantly altering its activity, leading to functional mutant proteases that are resistant to reversible inhibitors. An alternative approach to the inactivation of HIV-PR is thus based on irreversible inhibitors; these should be less sensitive to mutations because a reduced affinity will lower the equilibrium concentration of the enzyme-inhibitor complex and slow down, but not necessarily stop, the reaction between the inhibitor and the enzyme leading to its inactivation.

Targeting the active site of aspartyl proteases for irreversible inhibition requires a potent electrophile, which must be able to alkylate the carboxylate group notwithstanding its inherent low nucleophilicity. At the same time the electrophile must be sufficiently selective not to interact with other potential targets. The combination of these two contrasting properties makes the design of efficient irreversible inhibitors a challenging problem.

It was shown some years ago that 3-(4-nitro)phenoxy-1,2-epoxypropane (EPNP, **1**), a general inhibitor of aspartyl proteases, irreversibly inactivates HIV-PR by alkylating the active site Asp25 ($K_{inact} = 11 \text{ mM}$).^{15,16} Not surprisingly, however, this activated epoxide is not selective and its reaction with DNA has been recently demonstrated.¹⁷ In 1994, Ortiz de Montellano and coworkers reported the irreversible inhibition of HIV-PR by epoxides and α,β -unsaturated ketones structurally derived from haloperidol.¹⁸ The same group later showed that only the haloperidol derived epoxides selectively alkylate one of the active-site aspartate residues, while reaction of the protease with the more reactive enones or ynones resulted in alkylation of cysteine residues (Cys67 and Cys95) and the N-terminal proline in both subunits.¹⁹ Haloperidol derived epoxides include **2** ($K_{inact} = 521 \text{ } \mu\text{M}$)¹⁶ and the simplified inhibitor **3** ($K_{inact} = 65 \text{ } \mu\text{M}$).¹⁹

Based on these reports, a number of designed inhibitors have been described in which the epoxide is incorporated in a peptide or peptidomimetic structure in order to improve the inhibitor's binding affinity;²⁰⁻²⁴ some examples (**4-6**) are reported in Figure 1. A mechanism for the alkylation of the catalytic aspartate, involving hydrogen-bond assisted ring opening of the bound epoxide has also been proposed.^{25,26} These studies clearly indicate the potential of epoxides as selective, irreversible HIV-PR inhibitors and led us to investigate novel peptidomimetic structures which combine an epoxide ring with flanking residues derived from

our previous experience on competitive HIV-PR inhibitors.²⁷⁻²⁹ Herein we describe the design, synthesis and preliminary evaluation of HIV-PR inhibitors based on a novel epoxyalcohol motif.

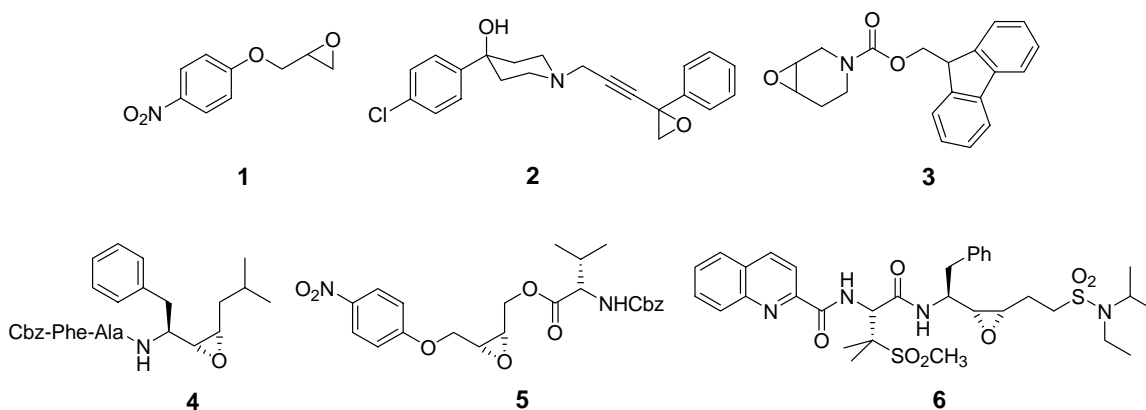
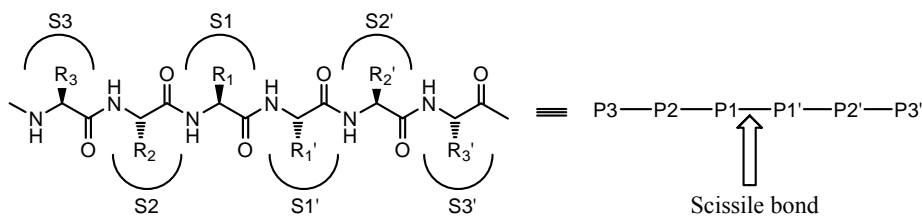


Figure 1. Epoxide-based irreversible inhibitors of HIV-PR.

Results and Discussion

Design. Structural studies of complexes of HIV-PR with peptide substrates and peptidomimetic inhibitors have allowed the conclusion that in the extended conformation in which the substrate is recognized by the protease, essential binding interactions are established between three residues on each side of the scissile bond and corresponding subsites of the enzyme.^{8,9,13} According to standard nomenclature, the residues are designated P3, P2, P1, P1', P2', P3' while the corresponding subsites are referred to as S3- -S3'.



Efficient peptidomimetic inhibitors can be obtained by replacing the scissile peptide bond P1-P1' with a non-scissile dipeptide isostere Ψ [P1-P1'] and optimizing the P3- -P3' residues for binding to the corresponding subsites.^{9,13,14} Hydroxyethylene and dihydroxyethylene dipeptide isosteres³⁰ are widely used in the design of aspartic protease inhibitors as the enzyme-inhibitor complex is stabilized by interactions between the catalytic aspartates and the hydroxy groups of the isostere, which are believed to mimic the tetrahedral transition state for amide hydrolysis.^{9,13} We thus decided to incorporate a hydroxy group in the design of our inhibitors, whose function is to bind the active site carboxylates and hold the epoxide in the correct position for irreversible alkylation (Figure 2).

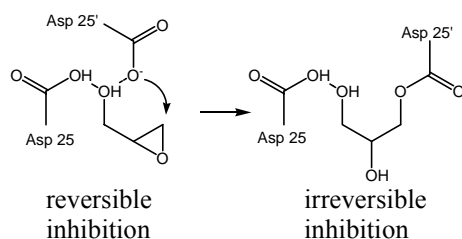


Figure 2. Proposed mechanism of inactivation of HIV-PR by epoxyalcohol inhibitors.

Epoxyalcohol inhibitors **7** and **8** (Figure 3) were designed starting from the structure of hexameric inhibitor **PV**, based on a dihydroxyethylene isostere, which inhibits HIV-PR with an IC_{50} of 15 nM.²⁸ This C_2 -symmetric inhibitor has Phe in P1 and P1', an arrangement that has been shown to be most effective for HIV-PR inhibition,^{9,13,14} Val in P2, P2' and is capped with phenoxyacetic acid (Poa) in both P3 and P3'. The latter residue has a low molecular weight and reduced peptide character while preserving hydrophobic interactions with the S3, S3' subsites. In **7** an epoxide ring is grafted on the P3--P1 portion of **PV** so as to generate an epoxyalcohol with the same configuration as in the central diol of **PV**. An aminomethyl group on the *trans* epoxide ring allows the introduction of a second Poa residue in a position intermediate between P1' and P2'; inhibitor **7** is thus expected to interact with the S3, S2, S1 and S1'/S2' subsites of the protease. While hexameric pseudopeptides are optimal for binding to the protease's active site, we have shown that pentameric and even tetrameric structures based on hydroxyethylene²⁹ and dihydroxyethylene isosteres²⁸ still provide sufficient binding interactions to inhibit the protease with IC_{50} values in the nM to μ M range. Since low molecular weight and reduced peptide character are important for the inhibitor's pharmacological properties, we decided to synthesize also the simplified epoxyalcohol **8**, in which P3 has been omitted and Val is replaced by Poa in P2.

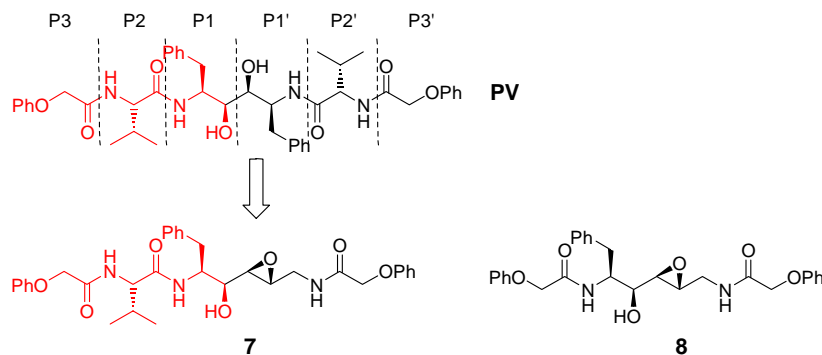
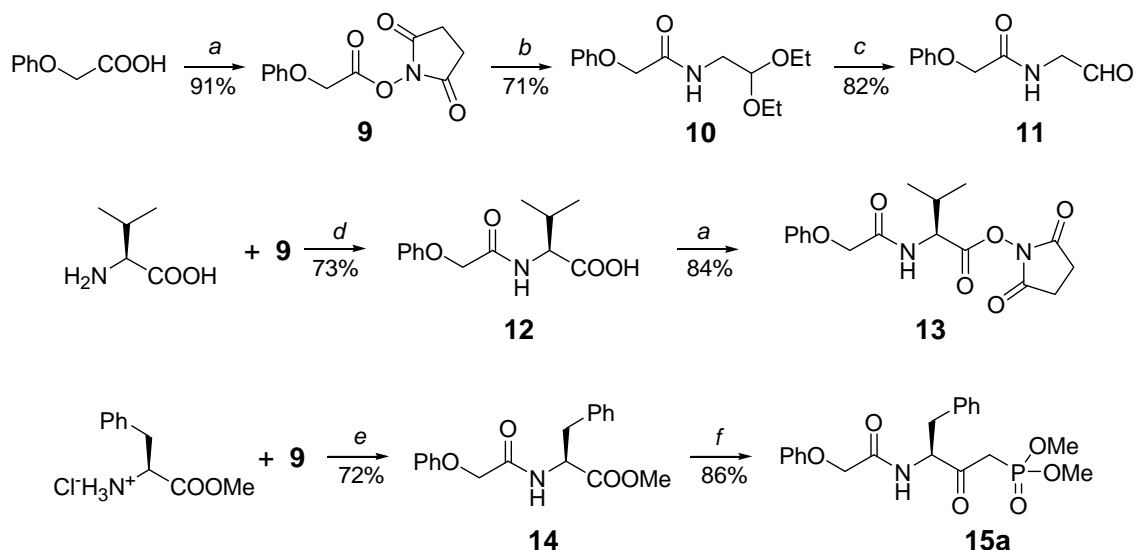


Figure 3. HIV-PR inhibitor **PV** and epoxyalcohol inhibitors **7**, **8**. The P3-P1 portion common to both **PV** and **7** is highlighted in red.

Synthesis. The approach to epoxyalcohols **7**, **8** started with the synthesis of precursors **11**, **13**, **15a** (Scheme 1). Phenoxyacetic acid was activated with N-hydroxysuccinimide (NHS) and condensed with aminoacetaldehyde diethyl acetal giving the protected phenoxyacetamido acetaldehyde **10**. Hydrolysis of the latter gave the required aldehyde **11**. Condensation of Poa-Osu **9** with L-valine gave phenoxyacetamido valine **12** (Poa-Val) which, in turn, was condensed with NHS to give the activated ester **13** (Poa-Val-Osu). The methyl ester of phenoxyacetyl phenylalanine **14** was obtained from the methyl ester of the amino acid and Poa-Osu **9** and then converted into the phosphonoketone **15a** by reaction with an excess of lithiated dimethyl methylphosphonate.³¹

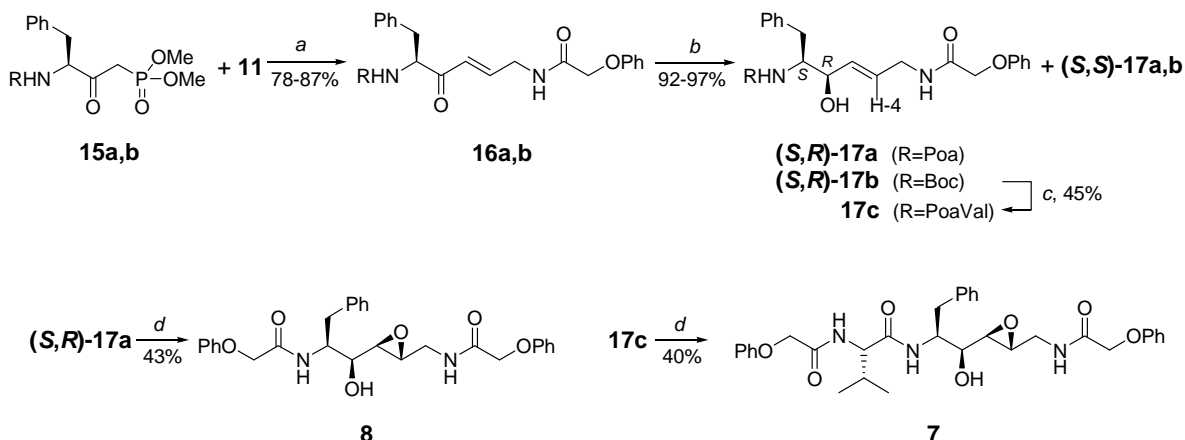


a- NHS, DCC, dioxane, 25 °C; *b*- 2,2-diethoxyethylamine, CH₂Cl₂, 25 °C; *c*- TFA, CH₂Cl₂/H₂O, 25 °C; *d*- dioxane/H₂O, NaOH, then HCl 25 °C; *e*- NEt₃, CH₂Cl₂, 25 °C; *f*- LiCH₂P(O)(OMe)₂, THF, -78 °C.

Scheme 1. Synthesis of precursors.

The next steps (Scheme 2) follow the general approach previously described³² for the synthesis of diaminiol dipeptide isosteres. Thus, Horner-Emmons olefination of phosphonoketone **15a** with the aldehyde **11**, under the conditions described by Mikolajczik,³³ gave the *E* enone **16a** in 78 % yield (Scheme 2). The *E* geometry of the double bond was confirmed by the 16 Hz coupling constant between the vinyl protons in the ¹H NMR spectrum of **16a**. Similarly, the N-Boc enone **16b**, was obtained from **11** and the corresponding Boc-protected phosphonoketone **15b**.³² The enones **16** were then reduced with sodium borohydride in methanol, at 0 °C, giving the allylic alcohols **17a,b** as 3 : 1 mixtures of diastereoisomers. Earlier work had allowed to establish that, in the NMR of allylic alcohols closely related to **17**,^{32,34} the H-4 vinylic proton of the *S,R* isomer (Scheme 2) always resonates at higher field than the

corresponding proton of the *S,S*-isomer. A similar situation has also been observed in other allylic alcohols derived from phenylalanine.³⁵ From the chemical shift of this diagnostic proton it was thus possible to assign the *S,R* and *S,S* configurations to the major and minor isomers of alcohols **17a,b**, respectively.



a- K_2CO_3 , EtOH, 25 °C; *b*- $NaBH_4$, MeOH, 0 °C; *c*- TFA, CH_2Cl_2 , then **13**, NEt_3 , CH_2Cl_2 , 25 °C; *d*- *m*-CPBA, $NaHCO_3$, CH_2Cl_2 .

Scheme 2. Synthesis of epoxyalcohol inhibitors.

The stereoselectivity of the reduction of enones **16** is consistent with chelation control by the metal or by the boron atom which might bind the carbamate nitrogen as suggested by Hoffmann et al.³⁶ (Figure 4a). Alternatively, the same result could be explained with a Felkin-Anh model in which NHBoc is the *medium* group and benzyl is the *large* group³² (Figure 4b). Based on calculations of the volume of the two groups, it has been recently suggested that NHBoc is larger than benzyl;³⁵ however, the bulky *t*-butyl group of NHBoc is four bonds away from the reaction center, to which it is connected by a linear, and relatively small, array of atoms. Thus the effectiveness of NHBoc in shielding the carbonyl from attack by the nucleophile may be overestimated by these calculations.

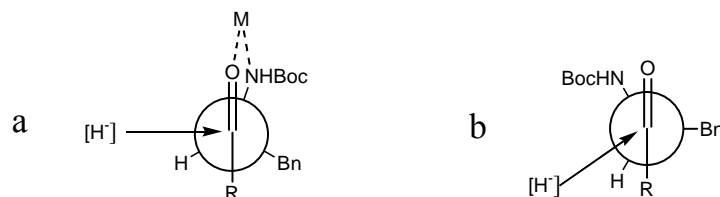


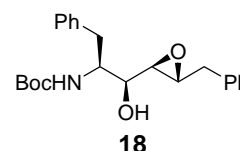
Figure 4. (a) Chelated and (b) Felkin–Anh models for borohydride reduction of aminoketones **16**.

Although the stereoselectivity of the reduction was not high, the diastereoisomers could be separated and obtained in pure form by column chromatography. The enantiomeric purity of alcohols (*S,R*)-**17a,b** was assessed from the ^1H and ^{13}C NMR spectra of the corresponding Mosher's esters, in which only one set of signals was observed, indicating that no epimerization had occurred at the carbon atom adjacent to the carbonyl up to this stage in the synthesis. The *N*-Boc alcohol (*S,R*)-**17b** was deprotected and the phenoxyacetyl valine residue was introduced by reaction with the activated ester **13** giving alcohol **17c** (Scheme 2). The final step is the epoxidation of allylic alcohols **17a,c** to give the required epoxides **8** and **7**. It is well known that peracid epoxidation of acyclic allylic alcohols is controlled by hydrogen bonding between the peracid and the hydroxy group of the substrate, leading to *syn*-epoxyalcohols³⁷ and high *syn* stereoselectivity was found in the epoxidation of a series of allylic alcohols structurally related to **17**;^{32,38} on this basis the *syn* configuration shown in scheme 2 was assigned to epoxyalcohols **7** and **8**.

Preliminary evaluation of the biological activity. Epoxyalcohols **7** and **8** were preliminarily assayed for their ability to competitively inhibit recombinant, wild-type HIV-PR using a standard activity test with a fluorogenic substrate.²⁸ IC_{50} values for **7**, **8**, for the reference inhibitor **PV** and for epoxyalcohol **18**, which was available from a previous investigation,³² are reported in Table 1. Data in the Table indicate that, on going from hexameric dihydroxyethylene inhibitor **PV** to tetrameric epoxyalcohol **7**, inhibition potency decreases by three orders of magnitude. This is not surprising considering that **7** can only interact with four of the six protease's subsites involved in substrate binding, as discussed earlier in the section on design. On the other hand, the IC_{50} of **7** is comparable with that of other tetrameric inhibitors based on hydroxyethylene²⁹ and dihydroxyethylene²⁸ isosteres, indicating that the more rigid *trans*-epoxide core of **7** does not interfere with binding to the protease's active site. Furthermore, very high affinity for the active site is not strictly required as **7** was designed as irreversible inhibitor. Removing the valine residue in P2 leads to a further increase in IC_{50} , as observed for **8**; an accurate evaluation of the inhibitory potency could not be obtained for this compound because of its poor solubility. Replacement of the Poa groups of **8** with Boc (in P2) and phenyl (P1'/P2') as in **18**, considerably decreases the inhibitor's activity, confirming the ability of this residue to provide strong interactions with the protease.³⁹

Table 1. IC_{50} values for HIV-PR inhibition

Inhibitor	IC_{50} (μM)	MW
7	39	589.69
8	~150	490.56
18	570	383.49
PV	0.015 ^a	770.97



^a From ref. 28.

Conclusions

A stereoselective approach originally developed for the synthesis of hydroxyethylene and dihydroxyethylene dipeptide isosteres has now been extended to the synthesis of HIV-PR inhibitors based on an epoxyalcohol core. Epoxyalcohols **7** and **8**, designed to irreversibly inhibit HIV-PR by alkylating the catalytic carboxylate groups, were synthesized by this approach in approximately 10% yield overall, starting from phenylalanine. Both epoxyalcohols are recognized by HIV-PR leading to inhibition with IC_{50} values in the μM range. As recognition by the binding site is a prerequisite for selective reaction of the epoxide with the active site carboxylates, the ability of epoxyalcohols **7** and **8** to competitively inhibit HIV-PR makes them good candidates for selective irreversible inhibition of the enzyme. Epoxyalcohol **7** is particularly promising in this respect with an IC_{50} value comparable to that of other tetrameric inhibitors of HIV-PR and a molecular weight only slightly exceeding the limit of 500 postulated by Lipinski's "rule of five".⁴⁰ A preliminary experiment indeed indicates that time-dependent inhibition is slowly taking place when HIV-PR is incubated with a given amount of epoxyalcohol **7**.

Experimental Section

General Procedures. Moisture-sensitive reactions were carried out in oven-dried vessels under a positive argon pressure. Tetrahydrofuran was distilled from sodium-benzophenone prior to use. Flash column chromatography was performed on Merck silica gel 60 (230-400 mesh); Merck silica gel 60_{F254} coated plastic sheets (0.25 mm) were used for TLC and developed with iodine and/or permanganate. Melting points were determined with a Büchi 510 open capillary apparatus and are uncorrected. Optical rotations were measured at 589 nm in methanol with a Perkin-Elmer 261 polarimeter fitted with a 10 cm cell. IR spectra were recorded as Nujol mulls, unless otherwise noted, on a JASCO 200 FT/IR spectrophotometer. ¹H NMR spectra (400 MHz) and ¹³C NMR spectra (100.4 MHz) were recorded for CDCl₃ solutions containing tetramethylsilane as an internal standard, on a Jeol EX400 spectrometer. Electrospray ionization mass spectra (ESI-MS) were obtained on a SCIEX Perkin-Elmer API1 spectrometer and electron impact mass spectra (EI-MS) were obtained on a VG 7070 spectrometer at the interdepartmental centre for mass spectrometry of the University of Trieste. CHN analyses were obtained on a Carlo Erba 1106 elemental analyzer. Diastereomeric ratios were measured by NMR.

1-[(Phenoxyacetyl)oxy]pyrrolidine-2,5-dione (Poa-Osu, **9).** Dicyclohexylcarbodiimide (DCC, 6.19 g, 30 mmol) was added in small portions, at 5 °C, to a stirred solution of phenoxyacetic acid (4.56 g, 30 mmol) and N-hydroxysuccinimide (NHS, 3.45 g, 0.30 mmol) in 80 mL dry dioxane. The resulting mixture was stirred at room temperature for 2 h, dicyclohexylurea was filtered off and the resulting solution was evaporated *in vacuo*. The residue was taken up in ethyl acetate and

the mixture was filtered again to eliminate residual dicyclohexylurea. The solvent was then evaporated giving crude **9** (6.8 g, 91%), m.p. 76–79 °C which was used without further purification. IR: cm^{-1} 1790, 1730 and 1700 (C=O). NMR: δ_{H} 2.83 (s, 4H), 4.97 (s, 2H), 6.95 (d, 2H, $^3J = 8.2$ Hz), 7.03 (t, 1H, $^3J = 7.3$ Hz), 7.32 (m, 2H); δ_{C} 25.5, 63.2, 114.7, 122.4, 129.7, 157.1, 164.6, 168.7.

N-(2,2-Diethoxyethyl)-2-phenoxyacetamide (10). Poa-Osu (**7**, 5.63 g, 22.6 mmol) and 2,2-diethoxyethylamine (3.28 mL, 22.6 mmol) in 50 mL dry dichloromethane were kept at room temperature for 2 h upon which a clear solution was obtained. The solution was extracted with 10% aqueous HCl, saturated aqueous bicarbonate, water and saturated brine in the order (50 mL each), dried over anhydrous sodium sulfate and evaporated under reduced pressure giving the crude amide **10** (4.3 g, 71%) which was used without purification. IR (film): cm^{-1} 3438 (NH), 1673 (CO). NMR: δ_{H} 1.19 (t, 6H, $^3J = 7.0$ Hz), 3.50 (m, 4H), 3.69 (m, 3H), 4.50 (s, 2H), 6.84 (s, 1H, NH), 6.91 (d, 2H, $^3J = 8.4$ Hz), 7.01 (t, 1H, $^3J = 7.3$ Hz), 7.31 (m, 2H); δ_{C} 15.2, 41.3, 62.9, 67.2, 100.6, 114.5, 122.0, 129.6, 157.1, 168.2. ES-MS: m/z 268 MH^+ , 290 $[\text{M}+\text{Na}]^+$.

N-(2-Oxoethyl)-2-phenoxyacetamide (11). A solution of the crude acetal **10** (2.20 g, 8.23 mmol) in CH_2Cl_2 (100 mL) was vigorously stirred with 40 mL of 20% aqueous TFA until **10** could not be detected at the TLC (3–4 days). The organic phase was separated, extracted with saturated aqueous NaHCO_3 , water and sat. brine (50 mL each) and dried over anhydrous Na_2SO_4 . Evaporation of the solvent gave a residue which was triturated with ether giving the solid amide **11** (1.31 g, 82%), m.p. 88–90 °C. IR: cm^{-1} 3470 (NH), 1740 (CO). NMR: δ_{H} 4.28 (d, 2H, $^3J = 5.1$ Hz), 4.56 (s, 2H), 6.95 (d, 2H, $^3J = 8.1$ Hz), 7.04 (t, 1H, $^3J = 7.3$ Hz), 7.30 (m, 3H, ArH and NH), 9.68 (s, 1H); δ_{C} 49.5, 67.1, 114.6, 122.2, 129.7, 157.0, 168.7, 195.7. ES-MS: m/z 194 MH^+ . Anal. Calcd. for $\text{C}_{10}\text{H}_{11}\text{NO}_3$ (193.20): C 62.2; H 5.74; N 7.28. Found: C 62.4; H 5.79; N 7.29.

N-(Phenoxyacetyl)-L-valine (12). L-valine (2.1 g, 18 mmol) in 0.7 M aqueous NaOH (25 mL) was added dropwise to a solution of ester **9** in 50 mL dioxane and the reaction mixture was kept at room temperature for 1 h. The solution was acidified to pH 2 with conc. HCl and the acid **12** precipitated upon cooling (3.31 g, 73%); the crude product was crystallized from ethyl acetate, m.p. 128–130 °C. $[\alpha]_{\text{D}}^{25} = -17$ (c 0.2). IR: cm^{-1} 3372 and 3345 (NH); 1712 and 1625 (C=O). NMR: δ_{H} 0.85 (d, 3H, $^3J = 7.0$ Hz), 0.89 (d, 3H, $^3J = 6.8$ Hz), 2.20 (m, 1H), 4.51 (m, 2H), 4.57 (m, 1H), 6.88 (d, 2H, $^3J = 8.6$ Hz), 6.95 (t, 1H, $^3J = 7.3$), 7.08 (d, 1H, NH, $^3J = 9.0$ Hz), 7.22 (m, 2H), 9.82 (s, 1H, COOH); δ_{C} 17.5, 18.9, 31.0, 56.7, 67.1, 114.8, 122.2, 129.7, 157.0, 169.1, 175.0. EI-MS: m/z 251 (M^+ , 28%), 206 (28), 151 (60), 107 (98), 94 (100). Anal. Calcd. for $\text{C}_{13}\text{H}_{17}\text{NO}_4$ (251.28): C 62.1; H 6.80; N 5.59. Found: C 62.6; H 7.01; N 5.77.

2,5-Dioxopyrrolidin-1-yl N-(phenoxyacetyl)-L-valinate (13). The acid **12** (1.5 g, 5.97 mmol), NHS (0.69 g, 5.97 mmol) and DCC (1.23 g, 5.97 mmol), as described for the synthesis of **9**, gave the crude ester **13** (1.75 g, 84%) as a thick oil that was used without purification. IR (film): cm^{-1} 3450, 3350 (NH); 1810, 1770, 1730, 1680 (C=O). NMR: δ_{H} 1.02 (d, 3H, $^3J = 5.8$ Hz), 1.04 (d, 3H, $^3J = 5.8$ Hz), 2.38 (m, 1H), 2.84 (m, 4H), 4.57 (s, 2H), 5.00 (dd, 1H, $^3J = 4.9, 9.0$ Hz), 6.95

(d, 2H, $^3J = 7.9$ Hz), 7.04 (m, 2H, ArH and NH), 7.3 (m, 2H); δ_C 17.3, 18.7, 25.6, 25.7, 31.6, 54.9, 67.2, 114.8, 122.3, 129.8, 157.0, 167.2, 168.2, 168.5.

Methyl *N*-(phenoxyacetyl)-*L*-phenylalaninate (14). A mixture of Poa-Osu **9** (6.00 g, 24 mmol), phenylalanine methyl ester hydrochloride (5.18 g, 24 mmol) and triethylamine (3.34 mL, 24 mmol) in dry CH_2Cl_2 (100 mL) was stirred at room temperature for 2 h. Workup as described for **9** gave the ester **14**, m.p. 53 – 55 °C (5.40 g, 72%). $[\alpha]_D^{25} -8.8$ (*c* 0.2). IR: cm^{-1} 3400 (NH), 1730 and 1710 (C=O). NMR: δ_H 3.15 (d, 2H), 3.70 (s, 3H), 4.51 (m, 2H), 4.97 (m, 1H) 6.87 (d, 2H, $^3J = 8.0$ Hz), 7.04 (m, 4H, ArH and NH), 7.29 (m, 5H); δ_C 37.8, 52.4, 55.1, 67.1, 114.6, 122.0, 127.1, 128.5, 129.1, 129.6, 135.4, 157.0, 167.9, 171.3. ES-MS: m/z 314 MH^+ ; 331 $[\text{M}+\text{NH}_4]^+$. Anal. Calcd. for $\text{C}_{18}\text{H}_{19}\text{NO}_4$ (315.37): C, 69.0; H, 6.11; N, 4.49. Found: C, 68.7; H, 6.20; N, 4.78.

Dimethyl {(3*S*)-2-oxo-3-[(phenoxyacetyl)amino]-4-phenylbutyl}phosphonate (15a). A 2.5 M solution of *n*-BuLi in hexane (67 mL, 170 mmol) was added dropwise, under an argon atmosphere, at –78 °C, to dimethyl methylphosphonate (18.1 mL, 170 mmol) in dry THF and the resulting solution was stirred at –78 °C for 20 min. A solution of ester **14** (10.5 g, 33 mmol) in dry THF (66 mL) was added and the reaction mixture was stirred for 2 h at –78 °C under argon. The mixture was poured into 150 mL of a 20% aqueous solution of citric acid and extracted with ethyl acetate (3 x 70 mL); the combined organic phases were extracted with sat. NaHCO_3 and brine and dried over an. sodium sulfate. Evaporation of the solvent gave the product as an oil which was used for the next step without purification (11.49 g, 86%). IR (film): cm^{-1} 3300 (NH), 1720 and 1670 (C=O), 1245 (P=O). NMR: δ_H 3.04 (dd, 1H, $^2J = 14.1$ Hz, $^3J = 8.4$ Hz), 3.07 (dd, 1H, $^2J_{\text{H-H}} = 14.1$ Hz, $^2J_{\text{H-P}} = 22.5$ Hz), 3.21 (dd, 1H, $^2J = 14.1$ Hz, $^3J = 5.5$ Hz), 3.24 (dd, 1H, $^2J_{\text{H-H}} = 14.1$ Hz, $^2J_{\text{H-P}} = 22.5$ Hz), 3.72 (d, 3H, $^3J_{\text{H-P}} = 11.4$ Hz), 3.73 (d, 3H, $^3J_{\text{H-P}} = 11.4$ Hz), 4.41 (d, 1H, $^2J = 15.0$ Hz), 4.43 (d, 1H, $^2J = 15.0$ Hz), 4.99 (m, 1H), 6.87 (d, 2H, $^3J = 8.6$ Hz), 7.01 (t, 1H, $^3J = 7.3$ Hz), 7.12 – 7.32 (m, 7H), 7.43 (d, 1H, NH, $^3J = 8.0$ Hz); δ_C 36.5, 38.6 (d, $^1J_{\text{C-P}} = 130.5$ Hz), 53.0 (d, $^2J_{\text{C-P}} = 6.4$ Hz), 53.1 (d, $^1J_{\text{C-P}} = 6.4$ Hz), 59.2, 67.0, 114.5, 122.0, 127.0, 128.6, 129.2, 129.7, 135.8, 157.1, 168.3, 199.9 (d, $^2J_{\text{C-P}} = 6.4$ Hz). ES-MS: m/z 406 MH^+ , 423 $[\text{M}+\text{NH}_4]^+$.

***N,N'*-(2*E*,5*S*)-4-Oxo-6-phenylhex-2-ene-1,5-diyl]bis(2-phenoxyacetamide) (16a).** Oven dried potassium carbonate (0.39 g, 2.8 mmol) was added in small portions, over 20 min, to a solution of phosphonate **15a** (1.15 g, 2.8 mmol) and aldehyde **9** (0.55g, 2.8 mmol) in absolute ethanol (25 mL) and the suspension was stirred at room temperature for 25 h. The solid was filtered off and the solution was neutralized with glacial acetic acid and evaporated to dryness under reduced pressure. The residue was partitioned between ethyl acetate (25 mL) and sat. sodium bicarbonate (25 mL) and the aqueous phase was extracted with ethyl acetate (25 mL). The combined organic phases were extracted with brine, dried over an. sodium sulfate and rotary evaporated giving the crude enone (1.15 g). Flash chromatography (ethyl acetate) gave pure **15a** (1.04 g, 78%). $[\alpha]_D^{25} -7.8$ (*c* 0.5). IR (film): cm^{-1} 3410 (NH), 1690, 1685 (C=O), 1660 (C=C). NMR: δ_H 3.03 (dd, 1H, $^2J = 13.9$ Hz, $^3J = 6.0$ Hz), 3.08 (dd, 1H, $^2J = 13.9$ Hz, $^3J = 6.9$ Hz), 4.11 (m, 2H), 4.46 (s, 2H), 4.54 (s, 2H), 5.11 (m, 1H), 6.13 (d, 1H, =CHCO, $^3J = 15.9$ Hz), 6.77–7.36 (m, 18 H); δ_C 37.7,

39.7, 56.8, 67.1, 114.5, 122.1, 126.6, 127.0, 128.5, 129.3, 129.7, 129.8, 135.4, 143.9, 157.0, 167.9, 168.3, 195.8. ES-MS: m/z 473 MH^+ , 495 $[M+Na]^+$.

tert-Butyl {(1S,3E)-1-benzyl-2-oxo-5-[(phenoxyacetyl)amino]pent-3-en-1-yl}carbamate (16b). Phosphonate **15b**³² (1.15 g, 3.1 mmol) and aldehyde **11** (0.60 g, 3.1 mmol), as described for **16a**, gave **16b** (87% after chromatography), m.p. 98 – 100 °C (from diisopropyl ether). $[\alpha]_D^{25} = -3.3$ (*c* 0.3). IR: cm^{-1} 3415 and 3340 (NH), 1690 (C=O), 1660 (C=C). NMR: δ_H 1.33 (s, 9H), 2.87 (dd, 1H, $^2J = 13.6$ Hz, $^3J = 5.7$ Hz), 2.97 (dd, 1H, $^2J = 13.6$ Hz, $^3J = 6.7$ Hz), 4.04 (s, 2H), 4.45 (s, 2H), 4.65 (m, 1H), 5.15 (d, 1H, \underline{NH} , $^3J = 6.8$ Hz), 6.06 (d, 1H, $=\underline{CHCO}$, $^3J = 15.7$ Hz), 6.66–7.29 (m, 12H); δ_C 28.1, 37.9, 39.6, 58.6, 67.1, 79.7, 114.5, 122.2, 126.7, 128.3, 129.3, 129.6, 129.7, 135.9, 143.3, 155.0, 157.0, 168.2, 196.1. ES-MS: m/z 439 MH^+ , 462 $[M+Na]^+$, 339 $[MH-Boc]^+$. Anal. Calcd. for $C_{25}H_{30}N_2O_5$ (438.52): C, 68.4; H, 6.89; N, 6.41. Found: C, 67.9; H, 6.70; N, 6.28.

***N,N'*-[(2E,4R,5S)-4-Hydroxy-6-phenylhex-2-ene-1,5-diyl]bis(2-phenoxyacetamide) (17a).** $NaBH_4$ (0.032 g, 0.85 mmol) was added in small portions, at 0 °C, to a well stirred solution of enone **16a** in MeOH (15 mL) and stirring was continued for 45 min at the same temperature. The mixture was neutralized with glacial acetic acid, the solvent was removed under reduced pressure and the residue was partitioned between ethyl acetate (40 mL) and saturated aqueous $NaHCO_3$ (40 mL). The aqueous phase was extracted with ethyl acetate (40 mL) and the combined organic phases were extracted with sat. brine and dried over an. sodium sulfate. Evaporation of the solvent under reduced pressure gave a 3 : 1 mixture of (4R,5S) and (4S,5S) diastereoisomers (0.39 g, 97%) which were separated by flash chromatography (eluant: ethyl acetate/diethyl ether 1:1). **(4R,5S)-17a**. m.p. 111 °C (from diisopropyl ether). $[\alpha]_D^{25} = -37.3$ (*c* 0.3). IR: cm^{-1} 3425 (NH, OH), 1700, 1680 (C=O), 1650 (C=C). NMR: δ_H 2.78 (dd, 1H, $^2J = 14.3$ Hz, $^3J = 9.0$ Hz), 2.97 (dd, 1H, $^2J = 14.3$ Hz, $^3J = 5.2$ Hz), 3.46 (bs, 1H, OH), 3.95 (t, 2H), 4.23 (m, 1H), 4.31 (m, 1H), 4.33 (d, 1H, $^2J = 15.2$ Hz), 4.43 (d, 1H, $^2J = 15.2$ Hz), 4.47 (s, 2H), 5.65 (dd, 1H, $^3J = 5.7$, 15.6 Hz), 5.75 (dt, 1H, $^3J = 5.4$, 15.6 Hz), 6.65 (d, \underline{NH} , $^3J = 8.0$ Hz), 6.72 (m, 1H, NH), 6.79 (d, 2H), 6.91 (d, 2H), 7.01 (m, 2H), 7.13 (d, 2H), 7.17–7.35 (m, 7H); δ_C 35.6, 40.2, 55.2, 67.2, 73.4, 114.6, 114.65, 122.1, 126.7, 128.4, 128.6, 129.1, 129.7, 129.8, 131.1, 137.1, 157.0, 168.1, 169.0. EI-MS: m/z 456 ($[M-H_2O]^+$ 1%), 107 (65), 94 (62), 77 (100). Anal. Calcd. for $C_{28}H_{30}N_2O_5$ (474.56): C, 70.9; H, 6.37; N, 5.93. Found: C, 70.6; H, 6.22; N, 5.92. **(4S,5S)-17a**: m. p. 128–130 °C (from diisopropyl ether). $[\alpha]_D^{25} = -50.6$ (*c* 0.34). IR: cm^{-1} 3390 (NH, OH), 1700, 1680 (C=O), 1650 (C=C). NMR: δ_H 2.88 (dd, 1H, $^2J = 13.5$ Hz, $^3J = 7.2$ Hz), 2.97 (dd, 1H, $^2J = 13.5$ Hz, $^3J = 5.0$ Hz), 2.90 (bs, 1H, OH), 3.87 (m, 2H), 4.16 (m, 2H), 4.31 (m, 1H), 4.37 (d, 1H, $^2J = 14.7$ Hz), 4.42 (s, 2H), 4.43 (d, 1H, $^2J = 14.7$ Hz), 5.56 (dd, 1H, $^3J = 5.1$, 15.6 Hz), 5.68 (dt, 1H, $^3J = 5.5$, 15.6 Hz), 6.65 (d, \underline{NH} , $^3J = 8.0$ Hz), 6.71 (m, 1H, NH), 6.8–6.92 (m, 5H), 7.15–7.35 (m, 9H); δ_C 37.6, 40.1, 54.6, 67.1, 67.3, 71.3, 114.6, 114.7, 122.1, 126.6, 127.5, 128.5, 129.2, 129.7, 129.8, 132.5, 137.6, 157.0, 157.2, 168.1, 168.6. EI-MS: m/z 456 ($[M-H_2O]^+$ 2%), 149 (100), 107 (28), 94 (40), 77 (50). Anal. Calcd. for $C_{28}H_{30}N_2O_5$ (474.56): C, 70.9; H, 6.37; N, 5.93. Found: C, 71.2; H, 6.30; N, 5.92.

tert-Butyl {(1*S*,2*R*,3*E*)-1-benzyl-2-hydroxy-5-[(phenoxyacetyl)amino]pent-3-en-1-yl} carbamate (17b). Borohydride reduction of enone **16b** (0.59 g, 1.3 mmol) gave a 3 : 1 mixture of (1*S*,2*R*) and (1*S*,2*S*) diastereoisomers (0.53 g, 92%) which were separated by flash chromatography (eluant: ether). **(1*S*,2*R*)-17b.** m.p. 129 °C (from diisopropyl ether). $[\alpha]_D^{25} = -41$ (*c* 0.29). IR: cm^{-1} 3470 (NH, OH), 1680 (C=O), 1645 (C=C). NMR: δ_H 1.28 (s, 9H), 2.64 (m, 1H), 2.73 (dd, 1H, $^2J = 14.2$ Hz, $^3J = 5.2$ Hz), 3.88 (m, 2H), (s, 2H), 4.13 (broad, 1H, OH), 4.40 (m, 1H), 4.42 (s, 2H), 4.70 (d, 1H, NH, $^3J = 8.2$ Hz), 5.59 (dd, 1H, $^3J = 5.7, 15.5$ Hz), 5.75 (dt, 1H, $^3J = 5.5, 15.5$ Hz), 6.69 (m, 1H, NH), 6.84 (d, 2H), 6.94 (t, 1H), 7.05-7.25 (m, 7H); δ_C 28.7, 35.9, 40.3, 56.4, 67.1, 73.8, 79.7, 114.6, 122.1, 126.3, 127.9, 128.4, 129.2, 129.7, 131.4, 137.7, 156.4, 157.0, 168.2. EI-MS: *m/z* 365 (20%), 274 (100), 151 (55). Anal. Calcd. for $\text{C}_{25}\text{H}_{32}\text{N}_2\text{O}_5$ (440.54): C, 68.1; H, 7.32; N, 6.38. Found: C, 68.1; H, 7.39; N, 6.46. **(1*S*,2*S*)-17b:** m.p. 99 – 101 °C (from diisopropyl ether). IR: cm^{-1} 3326 (NH, OH), 1676 (C=O), 1650 (C=C). NMR: δ_H 1.40 (s, 9H), 2.75-3.05 (m, 2H), 3.96 (m, 2H), 4.10 (broad, 1H, OH), 4.45 (m, 1H), 4.50 (s, 2H), 4.94 (d, 1H, NH, $^3J = 9.1$ Hz), 5.70 (m, 2H), 6.75-7.35 (m, 11H); δ_C 28.2, 36.1, 40.7, 56.6, 67.2, 74.3, 79.7, 114.9, 121.8, 126.8, 127.8, 128.4, 129.1, 129.7, 131.6, 137.9, 156.8, 157.0, 168.1. ES-MS: *m/z* 441 MH^+ , 458 $[\text{M}+\text{NH}_4]^+$, 341 $[\text{MH}-\text{Boc}]^+$. Anal. Calcd. for $\text{C}_{25}\text{H}_{32}\text{N}_2\text{O}_5$ (440.54): C, 68.1; H, 7.32; N, 6.38. Found: C, 68.3; H, 7.45; N, 6.33.

***N*²-(Phenoxyacetyl)-*N*¹-[(1*S*,2*R*,3*E*)-1-benzyl-2-hydroxy-5-(phenoxyacetyl)amino]pent-3-en-1-yl]-L-valinamide (17c).** N-Boc protected alcohol **(1*S*,2*R*)-17b** (405 mg, 0.92 mmol) in 2 mL dichloromethane was deprotected with trifluoroacetic acid (1 mL) for 10 min. The solvent was evaporated under reduced pressure and the residue was partitioned between dichloromethane (10 mL) and sat. NaHCO_3 (10 mL). The organic phase was extracted with sat. brine and dried over an. sodium sulfate. To the solution containing the deprotected amine were added the activated ester **13** (321 mg, 0.92 mmol) and triethylamine (132 μL , 0.92 mmol) and the reaction mixture was kept at room temperature for 12 h. The solution was extracted with sat. NaHCO_3 and brine, dried over an. sodium sulfate and the solvent was evaporated under reduced pressure. The residue was triturated with ethyl acetate giving the alcohol **17c** (235 mg, 45%); m.p. 177-179 °C (from ethyl acetate). $[\alpha]_D^{25} = -30.2$ (*c* 0.2). IR: cm^{-1} 3330 (NH, OH), 1665 (C=O), 1640 (C=C). NMR: δ_H 0.64 (d, 3H, $^3J = 6.1$ Hz), 0.77 (d, 3H, $^3J = 6.1$ Hz), 1.84 (broad, 1H, OH), 2.02 (m, 1H), 2.60 (dd, 1H, $^2J = 13.6$ Hz, $^3J = 9.7$ Hz), 2.80 (dd, 1H, $^2J = 13.6$ Hz, $^3J = 5$ Hz), 3.91 (s, 2H), 4.05-4.30 (m, 3H), 4.32 (d, 1H, $^3J = 15.0$ Hz), 4.40 (d, 1H, $^3J = 15.0$ Hz), 4.44 (s, 2H), 5.59 (dd, 1H, $^3J = 4.6, 15.2$ Hz), 5.73 (dt, 1H, $^3J = 5.0, 15.4$ Hz), 6.34 (m, 1H), 6.7-7.3 (m, 17H); δ_C 17.6, 19.3, 30.0, 35.7, 40.4, 55.5, 58.3, 66.9, 67.2, 76.7, 114.6, 122.1, 122.2, 126.6, 127.2, 128.4, 129.0, 129.2, 129.7, 129.8, 130.8, 137.4, 157.0, 157.1, 168.4, 168.6, 171.3. Anal. Calcd. for $\text{C}_{33}\text{H}_{39}\text{N}_3\text{O}_6$ (573.70): C, 69.1; H, 6.85; N, 7.35. Found: C, 69.0; H, 6.90; N, 7.29.

2,3-Anhydro-1,5,6-trideoxy-1,5-bis[(phenoxyacetyl)amino]-6-phenyl-L-glucitol (8). A solution of 60% *m*-CPBA (50 mg, 0.18 mmol) in dry CH_2Cl_2 (5 mL) was added to a stirred suspension of sodium hydrogen carbonate (15 mg, 0.18 mmol) in dichloromethane (5 mL) in which the alkene **(1*S*,2*R*)-17a** (70 mg, 0.15 mmol) had been previously dissolved. The reaction mixture was stirred at room temperature for 24 h, extracted with 5% aqueous sodium

metabisulfite, sat. bicarbonate and brine (5 mL each), and dried over an. sodium sulfate. The solvent was evaporated under reduced pressure giving the crude epoxide **8** (30 mg, 43%); m.p. 185 °C (dec., from ethyl acetate). $[\alpha]_D^{25} = -17.5$ (*c* 0.24). IR: cm^{-1} 3290 (NH, OH), 1648 (C=O). NMR: δ_H 1.7 (broad, 1H, OH), 2.84 (m, 1H), 2.97 (m, 2H), 3.08 (m, 1H), 3.45-3.65 (m, 3H), 4.36 (d, 1H, $^2J = 15.0$ Hz), 4.41 (m, 1H), 4.47 (s, 2H), 4.49 (d, 1H, $^2J = 15.0$ Hz), 6.7-7.3 (m, 17H); δ_C 35.7, 39.4, 53.1, 54.1, 57.8, 67.0, 67.1, 71.8, 114.5, 114.6, 122.1, 122.2, 126.8, 128.7, 129.3, 129.7, 129.8, 136.6, 157.0, 157.1, 168.8, 168.9. ES-MS: *m/z* 491 MH^+ , 513 $[\text{M}+\text{Na}]^+$. Anal. Calcd. for $\text{C}_{28}\text{H}_{30}\text{N}_2\text{O}_6$ (490.55): C, 68.6; H, 6.16; N, 5.71. Found: C, 68.2; H, 6.26; N, 5.95.

2,3-Anhydro-1,5,6-trideoxy-1-[(phenoxyacetyl)amino]-5-[[N-(phenoxyacetyl)-L-valyl]amino]-6-phenyl-L-glucitol (7). Epoxidation of **17c** (110 mg, 0.19 mmol) with 60% m-CPBA (66 mg, 0.23 mmol) and NaHCO_3 (19 mg, 0.23 mmol) gave **7** (45 mg, 40%); m.p. 145 °C (dec., ethyl acetate). $[\alpha]_D^{25} = -16.9$ (*c* 0.26). IR: cm^{-1} 3280 (NH, OH), 1650 (C=O). NMR: δ_H 0.61 (d, 3H, $^3J = 6.8$ Hz), 0.74 (d, 3H, $^3J = 6.8$ Hz), 1.68 (s, 1H, OH), 2.07 (m, 1H), 2.65-2.95 (m, 3H), 3.05 (m, 1H), 3.4-3.5 (m, 3H), 4.11 (m, 1H), 4.4 (m, 5H), 6.37 (d, 1H, NH, $^3J = 6.8$ Hz), 6.8-7.3 (m, 17H); δ_C 17.4, 19.3, 29.8, 36.0, 39.4, 53.7, 54.2, 57.6, 58.6, 67.1, 67.2, 71.9, 114.6, 114.7, 122.2, 122.3, 126.7, 128.6, 129.0, 129.8, 129.9, 137.1, 157.0, 157.1, 168.8, 168.9, 170.9. ES-MS: *m/z* 590 MH^+ , 612 $[\text{M}+\text{Na}]^+$. Anal. Calcd. for $\text{C}_{33}\text{H}_{39}\text{N}_3\text{O}_7$ (589.69): C, 67.2; H, 6.67; N, 7.13. Found: C, 67.4; H, 6.28; N, 7.15.

Enzyme assays. Recombinant HIV-1 protease was purchased from Bioczech (Prague, Czech Republic). The fluorogenic substrate Abz-Thr-Ile-Nle-Phe(NO₂)-Gln-Arg-NH₂ was from Bachem (Bubendorf, CH). The extent of inhibition of the activity of HIV-1 PR was evaluated as previously reported for compounds based on a dihydroxyethylene module.²⁸ In particular, assays were performed at pH 5.5 and 25°C in 400 mM NaCl, 1 mM EDTA, 1 mM DTT, 100 mM MES-NaOH buffer, in the presence of 30 μM fluorogenic substrate Abz-Thr-Ile-Nle-Phe(NO₂)-Gln-Arg-NH₂ and 10 nM HIV-PR.

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